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(21) International Application Number: PCT/US97/05780 (22) International Filing Date: 8 April 1997 (08.04.97) (30) Priority Data: 08/823,699 25 March 1997 (25.03.97) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 08/823,699 (CIP) Filed on 25 March 1997 (25.03.97) (71) Applicant (for all designated States except US): GELTEX PHARMACEUTICALS, INC. [US/US]; 303 Bear Hill Road, Waltham, MA 02154 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): GOLDBERG, Dennis, I. [US/US]; 109 Bent Road, Sudbury, MA 01776 (US). BURKE, Steven, K. [US/US]; 82 Willis Road, Sudbury, MA 01776 (US). MANDEVILLE, W., Harry, III [US/US]; 7 Pillings Pond Road, Lynnfield, MA 01940 (US). HOLMES-FARLEY, Stephen, Randall [US/US]; 20 Norfolk Road, Arlington, MA 02174 (US). WHITESIDES,		George, M. [US/US]; 124 Grasmere Street, Newton, MA 02158 (US). (74) Agents: BROOK, David, E. et al.; Hamilton, Brook, Smith & Reynolds, P.C., Two Militia Drive, Lexington, MA 02173 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published With international search report.
(54) Title: PHOSPHATE-BINDING POLYMERS COMBINED WITH A CALCIUM SUPPLEMENT FOR ORAL ADMINISTRATION		
(57) Abstract Phosphate-binding polymers are provided for removing phosphate from the gastrointestinal tract. The polymers are orally administered, and are useful for the treatment of hyperphosphatemia.		

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PHOSPHATE-BINDING POLYMERS COMBINED WITH A CALCIUM
SUPPLEMENT FOR ORAL ADMINISTRATION

BACKGROUND OF THE INVENTION

Hyperphosphatemia frequently accompanies diseases associated with inadequate renal function,
5 hypoparathyroidism, and certain other medical conditions. Hyperphosphatemia is typically defined as possessing a serum phosphate levels of over about 6 mg/dL. The condition, especially if present over extended periods of time, leads to severe abnormalities in calcium and
10 phosphorus metabolism and can be manifested by aberrant calcification in joints, lungs, and eyes.

Therapeutic efforts to reduce serum phosphate include dialysis, reduction in dietary phosphate, and oral administration of insoluble phosphate binders to reduce
15 gastrointestinal absorption. Dialysis and reduced dietary phosphate are generally unsuccessful in adequately reversing hyperphosphatemia. Further difficulties in these therapeutic regimens include the invasive nature of dialysis and the difficulties in modifying dietary habits
20 in the latter therapy.

The oral administration of certain phosphate binders has also been suggested. Phosphate binders include calcium or aluminum salts. Calcium salts have been widely used to bind intestinal phosphate and prevent absorption. The
25 ingested calcium combines with phosphate to form insoluble calcium phosphate salts such as $\text{Ca}_3(\text{PO}_4)_2$, CaHPO_4 , or $\text{Ca}(\text{H}_2\text{PO}_4)_2$. Different types of calcium salts, including calcium carbonate, acetate (such as PhosLo[®] calcium acetate

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tablets), citrate, alginate, and ketoacid salts have been utilized for phosphate binding. Due to the high dosages generally required to treat hyperphosphatemia, these therapeutics frequently cause hypercalcemia in patients.

5 Hypercalcemia has been indicated in many serious side effects, such as cardiac arrhythmias, renal failure, and skin and visceral calcification.

Aluminum-based phosphate binders, such as Amphojel[®] aluminum hydroxide gel, have also been used for treating

10 hyperphosphatemia. These compounds complex with intestinal phosphate to form highly insoluble aluminum phosphate; the bound phosphate is unavailable for absorption by the patient. Prolonged use of aluminum gels leads to accumulations of aluminum, and often to aluminum toxicity,

15 accompanied by such symptoms as encephalopathy, osteomalacia, and myopathy.

Selected ion exchange resins have also been suggested for use in binding phosphate. Those tested include Dowex[®] anion-exchange resins in the chloride form, such as XF

20 43311, XY 40013, XF 43254, XY 40011, and XY 40012. These resins have several drawbacks for treatment of hyperphosphatemia, including poor binding efficiency, necessitating use of high dosages for significant reduction of absorbed phosphate.

25 Thus a need exists for improved phosphate treatment regimens which can be administered in acceptable dosage levels without resulting in many of the serious side effects discussed above.

SUMMARY OF THE INVENTION

30 The invention relates to the discovery that the co-administration of a calcium dietary supplement with a

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phosphate binding polymer, such as an anion exchange polymer, results in improved and/or synergistic phosphate binding properties. In general, the invention features a method of removing phosphate from a patient by ion
5 exchange, which involves oral administration of a therapeutically effective amount of a composition containing at least one phosphate-binding polymer with the administration of dietary calcium.

The invention provides an effective treatment for
10 decreasing the serum level of phosphate by binding phosphate in the gastrointestinal tract, without concomittantly increasing the absorption of any clinically undesirable materials, particularly aluminum or high dosages of calcium.

15 The polymer and the dietary calcium supplement can be administered individually or as components of a single composition, for example, a composition for oral administration comprising at least one phosphate-binding polymer and a calcium supplement.

20 Other features and advantages will be apparent from the following description of the preferred embodiments and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an illustration of the bound phosphate
25 relevant to solution phosphate concentration after a phosphate solution is treated with poly(dimethyl-aminopropylacrylamide).

Figure 2 is a graphic illustration of the phosphate concentration in fecal samples taken from rats fed with a
30 dietary supplement of a crosslinked polyallylamine and micro-crystalline cellulose (placebo).

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Figure 3 is a graphic illustration of the urinary phosphate concentration in rats similarly given a dietary supplement of a crosslinked polyallylamine and micro-crystalline cellulose (placebo).

5 Figure 4 illustrates the serum phosphate levels in end stage renal patients receiving RenaGel® phosphate binder (a crosslinked polyallylamine polymer) with (square) and without (diamond) a dietary calcium supplement during the length of the clinical trial. Figure 4 establishes that
10 the coadministration results in significantly reduced phosphate levels.

Figure 5 is a graph depicting the change in serum calcium in end stage renal failure patients receiving RenaGel® phosphate binder (a crosslinked polyallylamine
15 polymer) with (square) and without (diamond) a dietary calcium supplement during the length of the clinical trial.

Figure 6 illustrates the effect of RenaGel® phosphate binder with (square) and without (diamond) a dietary calcium supplement on serum parathyroid hormone.

20 Figure 7 illustrates the effect of RenaGel® phosphate binder with (square) and without (diamond) a dietary calcium supplement on serum calcium x phosphate product in end stage renal failure patients.

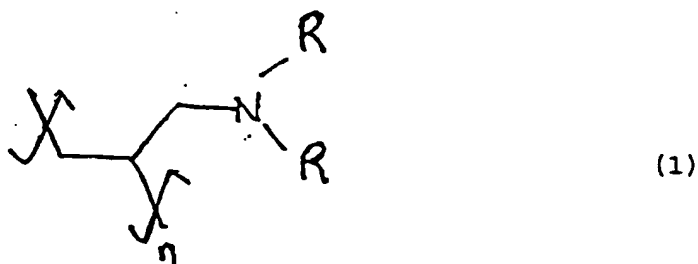
DESCRIPTION OF THE INVENTION

25 As described above, the invention relates to the unexpected discovery that the coadministration of a dietary calcium supplement and a phosphate-binding polymer (such as a anionic polymer) resulted in synergistic and superior serum phosphate control.

The Polymers

The polymers of the invention generally include phosphate-binding polymers, such as anion exchange polymers or resins. Preferred anion exchange polymers are hydrophilic anion exchange polymers or resins, particularly aliphatic amine polymers. The "amine" group can be present in the form of a primary, secondary or tertiary amine, quaternary ammonium salt, amidine, guanidine, hydrazine, or combinations thereof. The amine can be within the linear structure of the polymer (such as in polyethylenimine or a condensation polymer of a polyaminoalkane, e.g. diethylenetriamine, and a crosslinking agent, such as epichlorohydrin) or as a functional group pendant from the polymer backbone (such as in polyallylamine, polyvinylamine, poly(aminoethyl)-acrylate or poly(guanidinoacrylamide)).

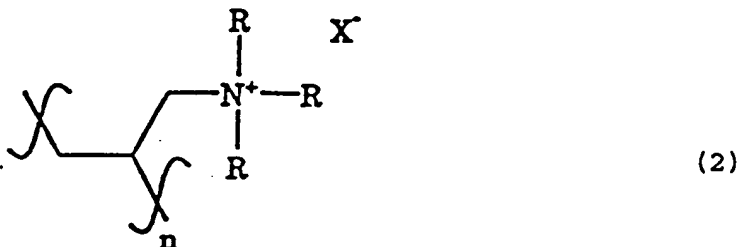
In one aspect, the polymer is characterized by a repeating unit having the formula



20 or a copolymer thereof, wherein n is an integer and each R,
independently, is H or a substituted or unsubstituted
alkyl, such as a lower alkyl (e.g., having between 1 and 5
carbon atoms, inclusive), alkylamino (e.g., having between
1 and 5 carbons atoms, inclusive, such as ethylamino) or
25 aryl (e.g., phenyl) group.

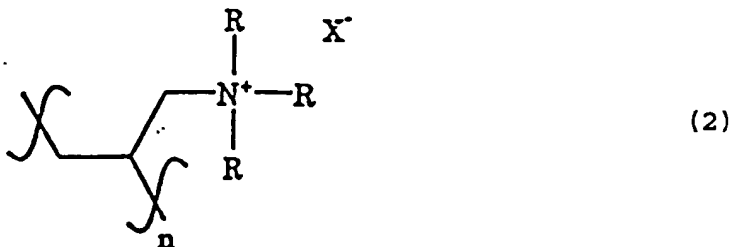
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In a second aspect, the polymer is characterized by a repeating unit having the formula



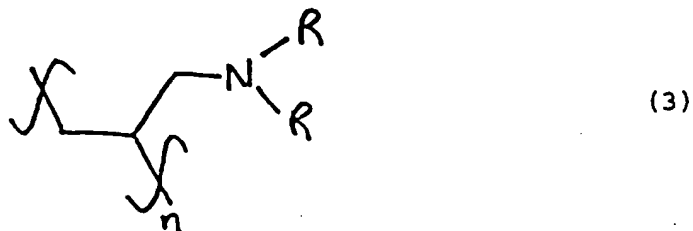
or a copolymer thereof, wherein n is an integer, each R ,
 5 independently, is H or a substituted or unsubstituted alkyl
 (e.g., having between 1 and 5 carbon atoms, inclusive),
 alkylamino (e.g., having between 1 and 5 carbons atoms,
 inclusive, such as ethylamino) or aryl (e.g., phenyl)
 group, and each X^- is an exchangeable negatively charged
 10 counterion.

One example of a copolymer according to the second
 aspect of the invention is characterized by a first
 repeating unit having the formula



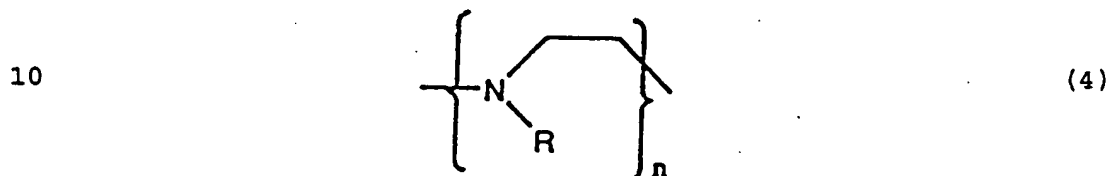
15 wherein n is an integer, each R , independently, is H or a
 substituted or unsubstituted alkyl (e.g., having between 1
 and 5 carbon atoms, inclusive), alkylamino (e.g., having
 between 1 and 5 carbons atoms, inclusive, such as
 ethylamino) or aryl group (e.g., phenyl), and each X^- is an
 20 exchangeable negatively charged counterion; and further
 characterized by a second repeating unit having the formula

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wherein each n , independently, is an integer and each R , independently, is H or a substituted or unsubstituted alkyl (e.g., having between 1 and 5 carbon atoms, inclusive),
 5 alkylamino (e.g., having between 1 and 5 carbons atoms, inclusive, such as ethylamino) or aryl group (e.g., phenyl).

In a fourth aspect, the polymer is characterized by a repeating unit having the formula



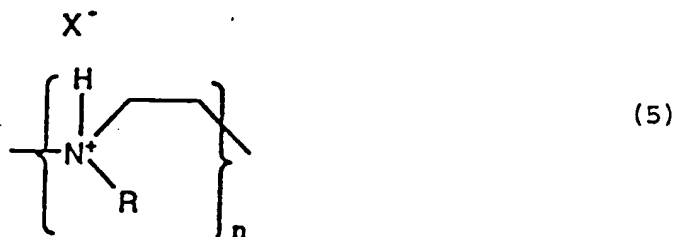
or a copolymer thereof, wherein n is an integer, and R is H or a substituted or unsubstituted alkyl (e.g., having between 1 and 5 carbon atoms, inclusive), alkylamino (e.g., having between 1 and 5 carbons atoms, inclusive, such as
 15 ethylamino) or aryl group (e.g., phenyl).

One example of a copolymer according to the second aspect of the invention is characterized by a first repeating unit having the formula

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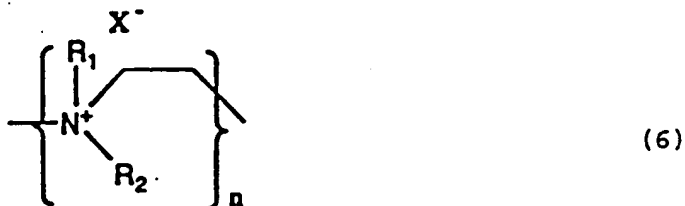


wherein n is an integer, and R is H or a substituted or unsubstituted alkyl (e.g., having between 1 and 5 carbon atoms, inclusive), alkylamino (e.g., having between 1 and 5 carbons atoms, inclusive, such as ethylamino) or aryl group (e.g., phenyl); and further characterized by a second repeating unit having the formula



wherein each n , independently, is an integer and R is H or a substituted or unsubstituted alkyl (e.g., having between 1 and 5 carbon atoms, inclusive), alkylamino (e.g., having between 1 and 5 carbon atoms, inclusive, such as ethylamino) or aryl group (e.g., phenyl).

In a fifth aspect, the polymer is characterized by a repeating group having the formula

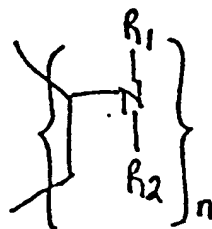


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or a copolymer thereof, wherein n is an integer, and each R_1 and R_2 , independently, is H or a substituted or unsubstituted alkyl (e.g., having between 1 and 5 carbon atoms, inclusive), and alkylamino (e.g., having between 1 and 5 carbons atoms, inclusive, such as ethylamino) or aryl group (e.g., phenyl), and each X^- is an exchangeable negatively charged counterion.

In one preferred polymer according to the fifth aspect of the invention, at least one of the R groups is a hydrogen atom.

In a sixth aspect, the polymer is characterized by a repeat unit having the formula



(7)

or a copolymer thereof, where n is an integer, each R_1 and R_2 , independently, is H, a substituted or unsubstituted alkyl group containing 1 to 20 carbon atoms, an alkylamino group (e.g., having between 1 and 5 carbons atoms, inclusive, such as ethylamino), or an aryl group containing 6 to 12 atoms (e.g., phenyl).

In a seventh aspect, the polymer is characterized by a repeat unit having the formula

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or a copolymer thereof, wherein n is an integer, each R_1 , R_2 and R_3 , independently, is H, a substituted or unsubstituted alkyl group containing 1 to 20 carbon atoms, an alkylamino group (e.g., having between 1 and 5 carbons atoms, inclusive, such as ethylamino), or an aryl group containing 6 to 12 atoms (e.g., phenyl), and each X^- is an exchangeable negatively charged counterion.

In each case, the R (including R, R_1 , R_2 and R_3) groups can carry one or more substituents. Suitable substituents include groups with an affinity for phosphate, such as anionic groups (e.g., quaternary ammonium groups) and amine groups (e.g., primary and secondary alkyl or aryl amines). Examples of other suitable substituents include groups which have an affinity for phosphate or are inert to phosphate, such as hydroxy, alkoxy, carboxamide, sulfonamide, halogen, alkyl, aryl, hydrazine, guanidine, urea, and carboxylic acid esters, for example.

The polymers are preferably crosslinked, in some cases by adding a crosslinking agent to the reaction mixture during or after polymerization. Examples of suitable crosslinking agents are diacrylates and dimethacrylates (e.g., ethylene glycol diacrylate, propylene glycol diacrylate, butylene glycol diacrylate, ethylene glycol dimethacrylate, propylene glycol dimethacrylate, butylene glycol dimethacrylate, polyethyleneglycol dimethacrylate, polyethyleneglycol diacrylate), methylene bisacrylamide, methylene bismethacrylamide, ethylene bisacrylamide, epichlorohydrin, epibromohydrin, toluene diisocyanate, ethylenebismethacrylamide, ethylidene bisacrylamide,

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divinyl benzene, bisphenol A dimethacrylate, bisphenol A diacrylate, 1,4-butanedioldiglycidyl ether, 1,2-ethanedioldiglycidyl ether, 1,3-dichloropropane, 1,2-dichloroethane, 1,3-dibromopropane, 1,2-dibromoethane, 5 succinyl dichloride, dimethylsuccinate, acryloyl chloride, or pyromellitic dianhydride.

The amount of crosslinking agent is typically between about 0.5 and about 75 weight%, and preferably between about 1 and about 25% by weight, based upon the combined 10 weight of crosslinking and monomer. In another embodiment, the crosslinking agent is present between about 2 and about 20% by weight of polymer.

In some cases the polymers are crosslinked after polymerization. One method of obtaining such crosslinking 15 involves reaction of the polymer with difunctional crosslinkers, such as epichlorohydrin, succinyl dichloride, the diglycidyl ether of bisphenol A, pyromellitic dianhydride, toluene diisocyanate, and ethylenediamine. A typical example is the reaction of poly(ethyleneimine) with 20 epichlorohydrin. In this example the epichlorohydrin (1 to 100 parts) is added to a solution containing polyethyleneimine (100 parts) and heated to promote reaction. Other methods of inducing crosslinking on already polymerized materials include, but are not limited 25 to, exposure to ionizing radiation, ultraviolet radiation, electron beams, radicals, and pyrolysis.

Examples of preferred crosslinking agents include epichlorohydrin, 1,4-butanedioldiglycidyl ether, 1,2-ethanedioldiglycidyl ether, 1,3-dichloropropane, 1,2- 30 dichloroethane, 1,3-dibromopropane, 1,2-dibromoethane, succinyl dichloride, dimethylsuccinate, toluene diisocyanate, acryloyl chloride, and pyromellitic dianhydride.

The negatively charged counterions, X^- , can be organic 35 ions, inorganic ions, or a combination thereof. The

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inorganic ions suitable for use in this invention include halide (especially chloride), carbonate, bicarbonate, sulfate, bisulfate, hydroxide, nitrate, persulfate and sulfite. Suitable organic ions include acetate, ascorbate, 5 benzoate, citrate, dihydrogen citrate, hydrogen citrate, oxalate, succinate, tartrate, taurocholate, glycocholate, and cholate.

In a preferred embodiment, the counterion does not have a detrimental side effect to the patient but rather is 10 selected to have a therapeutic or nutritional benefit to the patient.

POLYMER EXAMPLES

Candidate polymers were tested by stirring them in a phosphate containing solution at pH 7 for 3 hours. The 15 solution was designed to mimic the conditions present in the small intestine.

	Solution Contents
	10-20 mM Phosphate
	80 mM Sodium Chloride
20	30 mM Sodium Carbonate

The pH was adjusted to pH 7, once at the start of the test and again at the end of the test, using either aqueous NaOH or HCl. After 3 hours the polymer was filtered off and the residual phosphate concentration in the test 25 solution was determined spectrophotometrically. The difference between the initial phosphate concentration and the final concentration was used to determine the amount of phosphate bound to the polymer. This result is expressed in milliequivalents per gram of starting polymer (meq/g).

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Table 1 below shows the results obtained for several polymers. Higher numbers indicate a more effective polymer.

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TABLE 1.

Polymer	Phosphate Bound (meq/g) *
Poly(allylamine/epichlorohydrin)	3.1
Poly(allylamine/butanediol diglycidyl ether)	2.7
Poly(allylamine/ethanediol diglycidyl ether)	2.3
Poly(allyltrimethylammonium chloride)	0.3
Poly(ethyleneimine)/acryloyl chloride	1.2
Polyethyleneimine "C"	2.7
Polyethyleneimine "A"	2.2
Poly(DET/EPI)	1.5
Polyethyleneimine "B"	1.2
Poly(dimethylaminopropylacrylamide)	0.8
Poly(PEH/EPI)	0.7
Poly(trimethylammoniomethyl styrene chloride)	0.7
Poly(pentaethylenhexaminemethacrylamide)	0.7
Poly(tetraethylenepentaminemethacrylamide)	0.7
Poly(diethylenetriaminemethacrylamide)	0.5
Poly(triethylenetetraminemethacrylamide)	0.5
Poly(aminoethylmethacrylamide)	0.4
Poly(vinylamine)	0.4
Poly(MAPTAC)	0.24
Poly(methylmethacrylate/PEI)	0.2
Poly(dimethylethyleneimine chloride)	0.2
Poly(diethylaminopropylmethacrylamide)	0.1
Poly(guanidinoacrylamide)	0.1
Poly(guanidinobutylacrylamide)	0.1
Poly(guanidinobutylmethacrylamide)	0.1

* The values apply when the residual solution phosphate levels are ~5 mM.

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Table 2 shows results obtained using various other materials to bind phosphate.

TABLE 2.

Polymer	Phosphate Bound (meq/g) *
Calcium Chloride	4.0
Calcium Lactate	2.4
Ox-Absorb [®]	0.5
Maalox Plus [®]	0.3
Sephadex DEAE A-25, 40-125 m	0.2
Aluminum Hydroxide, Dried Gel	0.2

* The values apply when the residual solution phosphate levels are ~5 mM.

Table 3 shows results obtained for a variety of salts made from polyethyleneimine and organic and inorganic acids.

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TABLE 3.

Polymer	Phosphate Bound (meq/g) *
Poly(ethyleneimine sulfate A)	0.9
Poly(ethyleneimine sulfate B)	1.2
Poly(ethyleneimine sulfate C)	1.1
Poly(ethyleneimine sulfate D)	1.7
Poly(ethyleneimine tartrate A)	0.7
Poly(ethyleneimine tartrate B)	0.9
Poly(ethyleneimine tartrate C)	1.1
Poly(ethyleneimine ascorbate A)	0.55
Poly(ethyleneimine ascorbate B)	0.65
Poly(ethyleneimine ascorbate C)	0.9
Poly(ethyleneimine citrate A)	0.7
Poly(ethyleneimine citrate B)	1.0
Poly(ethyleneimine citrate C)	0.9
Poly(ethyleneimine succinate A)	1.1
Poly(ethyleneimine succinate B)	1.3
Poly(ethyleneimine chloride)	1.1

* The values apply when the residual solution phosphate levels are ~5 mM.

Oxabsorb[®] is an organic polymer that encapsulates calcium such that the calcium is available to bind to such ions as phosphate, but may not be released by the polymer and thus is not supposed to be absorbed by the patient.

5 The amount of phosphate bound by all of these materials, both polymers and inorganic gels, is expected to vary as the phosphate concentration varies. The graph in Figure 1 shows the relationship between the solution

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phosphate concentration and the amount of phosphate bound to poly(dimethylaminopropylacrylamide). Other polymers of the class are expected to show a similar relationship.

In an alternate type of test, the polymer was exposed
5 to an acidic environment prior to exposure to phosphate as might happen in a patient's stomach. The solid (0.1 g) was suspended in 40 mL of 0.1 M NaCl. This mixture was stirred for 10 minutes, and the pH was adjusted to 3.0 with 1 M HCl, and the mixture was stirred for 30 minutes. The
10 mixture was centrifuged, the supernatant decanted, and the solid resuspended in 40 mL of 0.1 m NaCl. This mixture was stirred for 10 minutes, the pH was adjusted to 3.0 with 1 M HCl, and the mixture was stirred for 30 minutes. The
15 mixture was centrifuged, the supernatant decanted, and the solid residue used in the usual phosphate assay. Results are shown in Table 4 for a variety of polymers and for aluminum hydroxide dried gel. In most cases the values for the amount of phosphate bound are higher in this test than in the usual assay.

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TABLE 4.

Polymer	Phosphate Bound (meq/g) *
Poly(ethyleneimine sulfate B)	1.2
Poly(ethyleneimine sulfate C)	1.3
Poly(ethyleneimine tartrate B)	1.3
Poly(ethyleneimine tartrate C)	1.4
Poly(ethyleneimine ascorbate B)	1.0
Poly(ethyleneimine ascorbate C)	1.0
Poly(ethyleneimine citrate B)	1.0
Poly(ethyleneimine citrate C)	1.3
Poly(ethyleneimine succinate A)	1.1
Poly(ethyleneimine succinate B)	1.3
Poly(ethyleneimine chloride)	1.4
Aluminum Hydroxide	0.7

* The values apply when the residual solution phosphate levels are ~5 mM.

Rat Dietary Phosphorus Excretion Model

Six 6-8 week old Sprague-Dawley rats were placed in metabolic cages and fed semi-purified rodent chow powder containing 0.28% inorganic phosphorus. The diets were
5 supplemented with 1.7% poly(allylamine/epichlorohydrin) or micro-crystalline cellulose; the animals served as their own controls by receiving cellulose or poly(allylamine/epichlorohydrin) in randomized order. The rats were fed ad libitum for three days to acclimate to the diet. Feces
10 excreted during the next 48 hours were collected, lyophilized, and ground into powder. The inorganic phosphate content was determined according to the method of

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Taussky and Shorr: Microdetermination of Inorganic Phosphate. One gram of powdered feces was burned to remove carbon, then ashed in a 600°C oven. Concentrated HCl was then added to dissolve the phosphorus. The phosphorus was
5 determined with ferrous sulfate-ammonium molybdate reagent. Intensity of the blue color was determined at 700 nm on a Perkin-Elmer spectrophotometer through a 1 cm cell.

The results are shown in Figure 2. Fecal phosphate concentration increased in all animals.

10 Urinary Phosphate Excretion in Partially Nephrectomized Rats

Sprague-Dawley rats, approximately 8 weeks old, were 75% nephrectomized. One kidney was surgically removed; approximately 50% of the renal artery flow to the
15 contralateral kidney was ligated. The animals were fed a semi-purified rodent chow containing 0.385% inorganic phosphorus and either 10% poly(allylamine/epichlorohydrin) or cellulose. Urine was collected and analyzed for phosphate content on specific days. Absorbed dietary
20 phosphate is excreted into the urine to maintain serum phosphate.

The results are shown in Figure 3. None of the animals became hyperphosphatemic or uremic, indicating that the residual kidney function was adequate to filter the
25 absorbed phosphate load. The animals receiving the poly(allylamine/epichlorohydrin) demonstrated a trend towards reduced phosphate excretion, indicative of reduced phosphate absorption.

SYNTHESES

30 Poly(allylamine hydrochloride) - To a 5 L, water jacketed reaction kettle equipped with 1) a condenser topped with a nitrogen gas inlet and 2) a thermometer and 3) a mechanical stirrer was added concentrated hydrochloric acid (2590 mL).

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- The acid was cooled to 5°C using circulating water in the jacket of the reaction kettle at 0°C. Allylamine (2362 mL; 1798 g) was added dropwise with stirring, maintaining a temperature of 5-10°C. After the addition was complete,
- 5 1338 mL of liquid was removed by vacuum distillation at 60-70°C. Azobis(amidinopropane) dihydrochloride (36 g) suspended in 81 mL water was added. The kettle was heated to 50°C under a nitrogen atmosphere with stirring for 24 hours. Azobis(amidinopropane) dihydrochloride (36 g)
- 10 suspended in 81 mL water was again added and the heating and stirring continued for an additional 44 hours. Distilled water (720 mL) was added and the solution allowed to cool with stirring. The liquid was added dropwise to a stirring solution of methanol (30 L). The solid was then
- 15 removed by filtration, resuspended in methanol (30 L), stirred 1 hour, and collected by filtration. This methanol rinse was repeated once more and the solid was dried in a vacuum oven to yield 2691 g of a granular white solid (poly(allylamine hydrochloride)).
- 20 Poly(allylamine/epichlorohydrin) - To a 5 gallon bucket was added poly(allylamine hydrochloride) (2.5 kg) and water (10 L). The mixture was stirred to dissolve and the pH was adjusted to 10 with a solid NaOH. The solution was allowed to cool to room temperature in the bucket and
- 25 epichlorohydrin (250 mL) was added all at once with stirring. The mixture was stirred gently until it gelled after about 15 minutes. The gel was allowed to continue curing for 18 hours at room temperature. The gel was then removed and put into a blender with isopropanol (about
- 30 7.5 L). The gel was mixed in the blender with about 500 mL isopropanol for ~3 minutes to form coarse particles and the solid was then collected by filtration. The solid was rinsed three times by suspending it in 9 gallons of water, stirring the mixture for 1 hour, and collecting the solid

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by filtration. The solid was rinsed once by suspending it in isopropanol (60 L), stirring the mixture for 1 hour, and collecting the solid by filtration. The solid was dried in a vacuum oven for 18 hours to yield 1.55 Kg of a granular, brittle, white solid.

Poly(allylamine/butanedioldiglycidyl ether) - To a 5 gallon plastic bucket was added poly(allylamine hydrochloride) (500 g) and water (2 L). The mixture was stirred to dissolve and the pH was adjusted to 10 with solid NaOH (142.3 g). The solution was allowed to cool to room temperature in the bucket and 1,4-butanedioldiglycidyl ether (130 mL) was added all at once with stirring. The mixture was stirred gently until it gelled after 4 minutes. The gel was allowed to continue curing for 18 hours at room temperature. The gel was then removed and dried in a vacuum oven at 75°C for 24 hours. The dry solid was ground and sieved for -30 mesh and then suspended in 6 gallons on water. After stirring for 1 hour the solid was filtered off and rinse process repeated twice more. The solid was rinsed twice in isopropanol (3 gallons), and dried in a vacuum oven at 50°C for 24 hours to yield 580 g of a white solid.

Poly(allylamine/ethanedioldiglycidyl ether) - To a 100 mL beaker was added poly(allylamine hydrochloride) (10g) and water (40 mL). The mixture was stirred to dissolve and the pH was adjusted to 10 with solid NaOH. The solution was allowed to cool to room temperature in the beaker and 1,2 ethanedioldiglycidyl ether (2.0 mL) was added all at once with stirring. The mixture was allowed to continue curing for 18 hours at room temperature. The gel was then removed and blended in 500 mL of methanol. The solid was filtered off and suspended in water (500 mL). After stirring for 1 hour the solid was filtered off and the rising process

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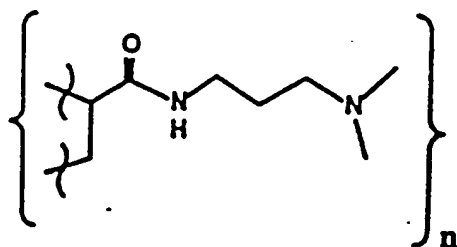
repeated. The solid was rinsed twice in isopropanol (400 mL), and dried in a vacuum oven at 50°C for 24 hours to yield 8.7 g of a white solid.

Poly(allylamine/dimethylsuccinate) - To a 500 mL round
5 bottom flask was added poly(allylamine hydrochloride)
(10 g), methanol (100 mL), and triethylamine (10 mL). The
mixture was stirred and dimethylsuccinate (1 mL) was added.
The solution was heated to reflux and stirring turned off
after 30 minutes. After 18 hours the solution was cooled
10 to room temperature and solid was filtered off and
suspended in water (1 L). After stirring for 1 hour the
solid was filtered off and the rinse process repeated twice
more. The solid was rinsed once in isopropanol (800 mL),
and dried in a vacuum oven at 50°C for 24 hours to yield
15 5.9 g of a white solid.

Poly(allyltrimethylammonium chloride) - To a 500 mL three
necked flask equipped with a magnetic stirrer, a
thermometer, and a condenser topped with a nitrogen inlet,
was added poly(allylamine) crosslinked with epichlorohydrin
20 (5.0 g), methanol (300 mL), methyl iodide (20 mL), and
sodium carbonate (50 g). The mixture was then cooled and
water was added to total volume of 2 L. Concentrated
hydrochloric acid was added until no further bubbling
resulted and the remaining solid was filtered off. The
25 solid was rinsed twice in 10% aqueous NaCl (1 L) by
stirring for 1 hour followed by filtration to recover the
solid. The solid was then rinsed three times by suspending
it in water (2 L), stirring for 1 hour, and filtering to
recover the solid. Finally, the solid was rinsed as above
30 in methanol and dried in a vacuum oven at 50°C for 18 hours
to yield 7.7 g of white granular solid.

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Poly(ethyleneimine)/acryloyl chloride - Into a 5 L three neck flask equipped with a mechanical stirrer, a thermometer, and an additional funnel was added polyethyleneimine (510 g of a 50% aqueous solution (equivalent to 255 g of dry polymer) and isopropanol (2.5 L). Acryloyl chloride (50 g) was added dropwise through the addition funnel over a 35 minute period, keeping the temperature below 29°C. The solution was then heated to 60°C with stirring for 18 hours. The solution was cooled and solid immediately filtered off. The solid was rinsed three times by suspending it in water (2 gallons), stirring for 1 hour, and filtering to recover the solid. The solid was rinsed once by suspending it in methanol (2 gallons), stirring for 30 minutes, and filtering to recover the solid. Finally, the solid was rinsed as above in isopropanol and dried in a vacuum over at 50°C for 18 hours to yield 206 g of light orange granular solid.

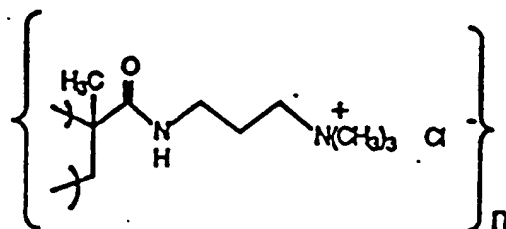


Poly(dimethylaminopropylacrylamide) - Dimethylaminopropylacrylamide (10 g) and methylene-bisacrylamide (1.1 g) were dissolved in 50 mL of water in a 100 mL three neck flask. The solution was stirred under nitrogen for 10 minutes. Potassium persulfate (0.3 g) and sodium metabisulfite (0.3 g) were each dissolved in 2-3 mL of

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water and then mixed. After a few seconds this solution was added to the monomer solution, still under nitrogen. A gel formed immediately and was allowed to sit overnight. The gel was removed and blended with 500 mL of isopropanol.

5 The solid was filtered off and rinsed three times with acetone. The solid white powder was filtered off and dried in a vacuum oven to yield 6.1 g.



Poly(Methacrylamidopropyltrimethylammoniumchloride) = [Poly

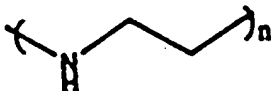
10 (MAPTAC)] - [3-(Methacryloylamino)propyl]trimethylammonium chloride (38 mL of 50% aqueous solution) and methylenebis-methacrylamide (2.2 g) were stirred in a beaker at room temperature. Methanol (10 mL) was added and the solution was warmed to 40°C to fully dissolve the bisacrylamide.

15 Potassium persulfate (0.4 g) was added and the solution stirred for 2 minutes. Potassium metabisulfite (0.4 g) was added and stirring was continued. After 5 minutes the solution was put under a nitrogen atmosphere. After 20

20 minutes the solution contained significant precipitate and the solution was allowed to sit overnight. The solid was washed three times with isopropanol and collected by filtration. The solid was then suspended in water 500 (mL) and stirred for several hours before being collected by centrifugation. The solid was again washed with water and

25 collected by filtration. The solid was then dried in a vacuum oven to yield 21.96 g.

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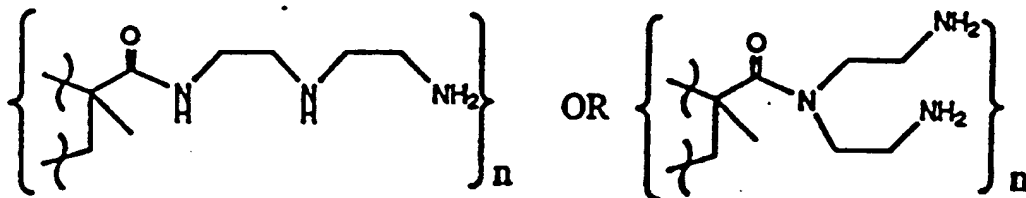


Poly(ethyleneimine) "A" - Polyethyleneimine (50 g of a 50% aqueous solution; Scientific Polymer Products) was dissolved in water (100 mL). Epichlorohydrin (4.6 mL) was
5 added dropwise. The solution was heated to 55°C for 4 hours, after which it had gelled. The gel was removed, blended with water (1 L) and the solid was filtered off. It was resuspended in water (2 L) and stirred for 10
10 minutes. The solid was filtered off, the rinse repeated once with water and twice with isopropanol, and the resulting gel was dried in a vacuum oven to yield 26.3 g of a rubbery solid.

Poly(ethyleneimine) "B" and Poly(ethyleneimine) "C" were made in a similar manner, except using 9.2 and 2.3 mL of
15 epichlorohydrin, respectively.

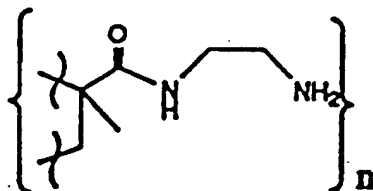
Poly(methylmethacrylate-co-divinylbenzene) - Methylmethacrylate (50 g) and divinylbenzene (5 g) and azobisisobutyronitrile (1.0 g) were dissolved in isopropanol (500 mL) and heated to reflux for 18 hours under a nitrogen
20 14 atmosphere. The solid white precipitate was filtered off, rinsed once in acetone (collected by centrifugation), once in water (collected by filtration and dried in a vacuum oven to yield 19.4 g.

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Poly(diethylenetriaminemethacrylamide) - Poly(methyl-methacrylate-co-divinylbenzene) (20 g) was suspended in diethylenetriamine (200 mL) and heated to reflux under a nitrogen atmosphere for 18 hours. The solid was collected by filtration, resuspended in water (500 mL), stirred 30 minutes, filtered off, resuspended in water (500 mL), stirred 30 minutes, filtered off, rinsed briefly in isopropanol, and dried in a vacuum oven to yield 18.0 g.

10

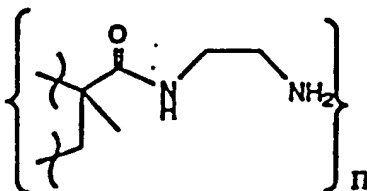


Poly(pentaethylenehexaminemethacrylamide), Poly(tetraethylenepentaminemethacrylamide), and Poly(triethylenetetraaminemethacrylamide) were made in a manner similar to poly(diethylenetriaminemethacrylamide) from pentaethylenehexamine, tetraethylenepentamine, and triethylenetetraamine, respectively.

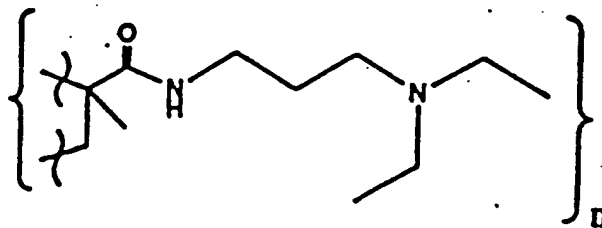
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Poly(methylmethacrylate/PEI) - Poly(methylmethacrylate-co-divinylbenzene) (1.0 g) was added to a mixture containing hexanol (9150 mL) and polyethyleneimine (15 g in 15 g water). The mixture was heated to reflux under nitrogen for 4 days. The reaction was cooled and the solid was filtered off, suspended in methanol (300 mL), stirred 1 hour, and filtered off. The rinse was repeated once with isopropanol and the solid was dried in a vacuum oven to yield 0.71 g.

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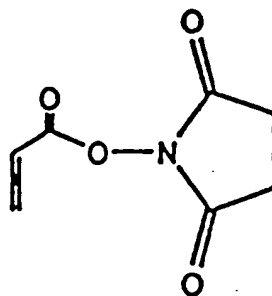


Poly(aminoethylmethacrylamide) - Poly(methylmethacrylate-co-divinylbenzene) (20 g) was suspended in ethylenediamine (9200 mL) and heated to reflux under a nitrogen atmosphere for 3 days. The solid was collected by centrifugation, washed by resuspending it in water (500 mL), stirring for 30 minutes, and filtering off the solid. The solid was washed twice more in water, once in isopropanol, and dried in a vacuum oven to yield 17.3 g.



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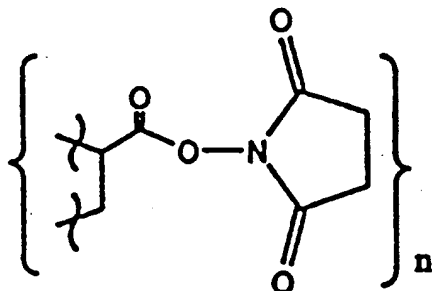
Poly(diethylaminopropylmethacrylamide) - Poly(methyl-methacrylate-co-divinylbenzene) (20 g) was suspended in diethylaminopropylamine (200 mL) and heated to reflux under a nitrogen atmosphere for 18 hours. The solid was
5 collected by filtration, resuspended in water (500 mL), filtered off, resuspended in water (500 mL), collected by filtration, rinsed briefly in isopropanol, and dried in a vacuum oven to yield 8.2 g.



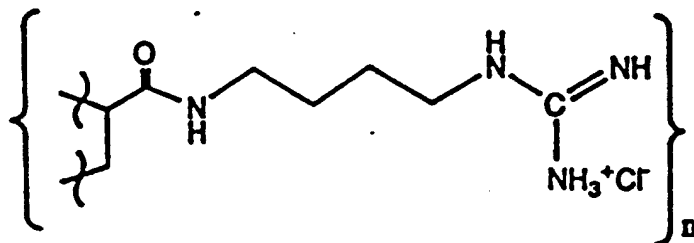
10 NHS-acrylate - N-Hydroxysuccinimide (NHS, 157.5 g) was dissolved in chloroform (2300 mL) in a 5 L flask. The solution was cooled to 0°C and acryloyl chloride (132 g) was added dropwise, keeping the temperature 2°C. After addition was complete, the solution was stirred for 1.5
15 hours, rinsed with water (1100 mL) in a separatory funnel and dried over anhydrous sodium sulfate. The solvent was removed under vacuum and a small amount of ethyl acetate was added to the residue. This mixture was poured into hexane (200 mL) with stirring. The solution was heated to
20 reflux, adding more ethyl acetate (400 mL). The insoluble NHS was filtered off, hexane (1 L) was added, the solution was heated to reflux, ethyl acetate (400 mL) was added, and the solution allowed to cool to <10°C. The solid was then filtered off and dried in a vacuum oven to yield 125.9 g.

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A second crop of 80 g was subsequently collected by further cooling.



Poly(NHS-acrylate) - NHS-acrylate (28.5 g), methylenebis-
 5 acrylamide (1.5 g) and tetrahydrofuran (500 mL) were mixed
 in a 1 L flask and heated to 50°C under a nitrogen
 atmosphere. Azobisisobutyronitrile (0.2 g) was added, the
 solution was stirred for 1 hour, filtered to remove excess
 N-hydroxysuccinimide, and heated to 50°C for 4.5 hours
 10 under a nitrogen atmosphere. The solution was then cooled
 and the solid was filtered off, rinsed in tetrahydrofuran,
 and dried in a vacuum oven to yield 16.1 g.

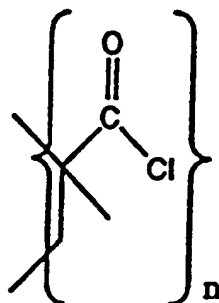


Poly(guanidinobutylacrylamide) - Poly(NHS-acrylate) (1.5 g)
 15 was suspended in water (25 mL) containing agmatine (1.5 g)
 which had been adjusted to pH 9 with solid NaOH. The
 solution was stirred for 4 days, after which time the pH

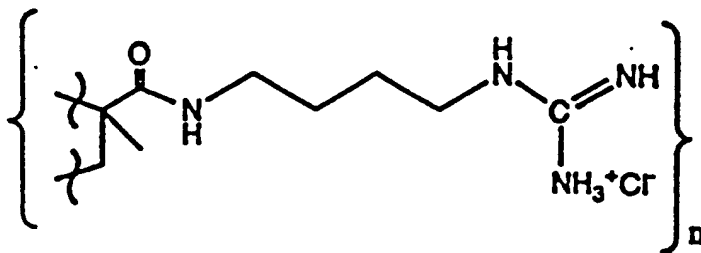
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had dropped to 6.3. Water was added to a total of 500 mL, the solution was stirred for 30 minutes and the solid was filtered off. The solid was rinsed twice in water, twice in isopropanol, and dried in a vacuum oven to yield 0.45 g.

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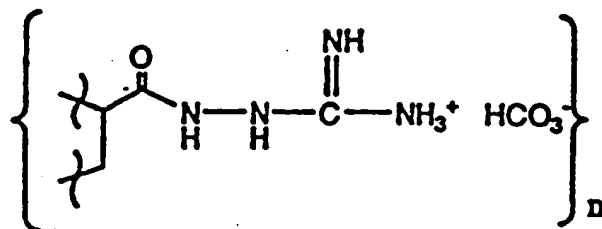
Poly(methacryloyl chloride) - Methacryloyl chloride (20 mL), divinyl benzene (4 mL of 80% purity), AIBN (0.4 g), and THF (150 mL) were stirred at 60°C under a nitrogen atmosphere for 18 hours. The solution was cooled and the solid was filtered off, rinsed in THF, then acetone, and dried in a vacuum oven to yield 8.1 g.



Poly(guanidinobutylmethacrylamide) - Poly(methacryloyl chloride) (0.5 g), agmatine sulfate (1.0 g), triethylamine (2.5 mL), and acetone (50 mL) were stirred together for 4

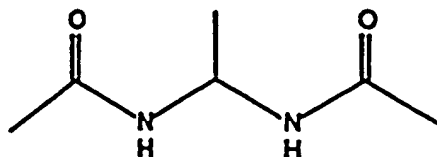
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days. Water (100 mL) was added and the mixture stirred for 6 hours. The solid was filtered off and washed by resuspending in water (500 mL), stirring for 30 minutes, and filtering off the solid. The wash was repeated twice
 5 in water, once in methanol, and the solid was dried in a vacuum oven to yield 0.41 g.



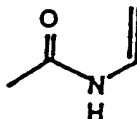
Poly(guanidinoacrylamide) - The procedure for poly-(guanidinobutylacrylamide) was followed substituting
 10 aminoguanidine bicarbonate (5.0 g) for the agmatine, yielding 0.75 g.

Poly(PEH/EPI) - Epichlorohydrin (1.5 g) was added dropwise to a solution containing pentaethylenhexamine (20 g) and water (100 mL), keeping the temperature between 65°C. The
 15 solution was stirred until it gelled and heating was continued for 4 hours (at 65°C). After sitting overnight at room temperature the gel was removed and blended with water (1 L). The solid was filtered off, water was added (1 L), and the blending and filtration were repeated. The
 20 gel was suspended in isopropanol and the resulting solid was collected by filtration and dried in a vacuum oven to yield 28.2 g.

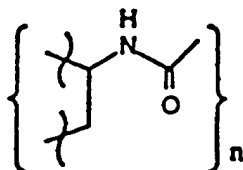


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Ethylidenebisacetamide - Acetamide (118 g), acetaldehyde (44.06 g), copper acetate (0.2 g), and water (300 mL) were placed in a 1 L three neck flask fitted with condenser, thermometer, and mechanical stirred. Concentrated HCl (34 mL) was added and the mixture was heated to 45-50°C with stirring for 24 hours. The water was then removed in vacuo to leave a thick sludge which formed crystals on cooling to 5°C. Acetone (200 mL) was added and stirred for a few minutes after which the solid was filtered off and discarded. The acetone was cooled to 0°C and solid was filtered off. This solid was rinsed in 500 mL acetone and air dried 18 hours to yield 31.5 g.

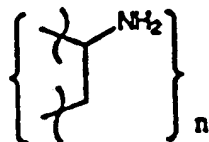


Vinylacetamide - Ethylidenebisacetamide (31.05), calcium carbonate (2 g) and celite 541 (2 g) were placed in a 500 mL three neck flask fitted with a thermometer, a mechanical stirrer, and a distilling head atop a vigroux column. The mixture was vacuum distilled at 35 mm Hg by heating the pot to 180-225°C. Only a single fraction was collected (10.8 g) which contained a large portion of acetamide in addition to the product (determined by NMR). This solid product was dissolved in isopropanol (30 mL) to form the crude solution used for polymerization.



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Poly(vinylacetamide) - Crude vinylacetamide solution (15 mL), divinylbenzene (1 g, technical grade, 55% pure, mixed isomers), and AIBN (0.3 g) were mixed and heated to reflux under a nitrogen atmosphere for 90 minutes, forming a solid precipitate. The solution was cooled, isopropanol (50 mL) was added, and the solid was collected by centrifugation. The solid was rinsed twice in isopropanol, once in water, and dried in a vacuum oven to yield 0.8 g.



Poly(vinylamine) - Poly(vinylacetamide) (0.79 g) was placed in a 100 mL one neck flask containing water 25 mL and concentrated HCl 25 mL. The mixture was refluxed for 5 days, the solid was filtered off, rinsed once in water, twice in isopropanol, and dried in a vacuum oven to yield 0.77 g. The product of this reaction (~0.84 g) was suspended in NaOH (46 g) and water (46 g) and heated to boiling (~140°C). Due to foaming the temperature was reduced and maintained at ~100°C for 2 hours. Water (100 mL) was added and the solid collected by filtration. After rinsing once in water the solid was suspended in water (500 mL) and adjusted to pH 5 with acetic acid. The solid was again filtered off, rinsed with water, then the isopropanol, and dried in a vacuum oven to yield 0.51 g.

Poly(trimethylammoniomethylstyrene chloride) is the copolymer of trimethylammoniomethylstyrene chloride and divinyl benzene.

Poly(DET/EPI) is the polymer formed by reaction of diethylenetriamine and epichlorohydrin.

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- Poly(ethyleneimine) Salts - Polyethyleneimine (25 g dissolved in 25 g water) was dissolved in water (100 mL) and mixed with toluene (1 L). Epichlorohydrin (2.3 mL) was added and the mixture heated to 60°C with vigorous
- 5 mechanical stirring for 18 hours. The mixture was cooled and the solid filtered off, resuspended in methanol (2 L), stirred 1 hour, and collected by centrifugation. The solid was suspended in water (2 L), stirred 1 hour, filtered off, suspended in water (4 L), stirred 1 hour, and again
- 10 filtered off. The solid was suspended in acetone (4 L) and stirred 15 minutes, the liquid was poured off, acetone (2 L) was added, the mixture was stirred 15 minutes, the acetone was again poured off, and the solid was dried in a vacuum oven to form intermediate "D".
- 15 Poly(ethyleneimine sulfate A) - Intermediate "D" (1.0 g) was suspended in water (150 mL), stirred 30 minutes, and partially neutralized with sulfuric acid (1.1 g). The mixture was stirred an additional 30 minutes, the solid was filtered off, resuspended in methanol (200 mL), stirred 5
- 20 minutes, filtered off, and dried in a vacuum oven.
- Poly(ethyleneimine sulfate B) - Intermediate "D" (1.0 g) was suspended in water (150 mL), stirred 30 minutes, and partially neutralized with sulfuric acid (0.57 g). The mixture was stirred an additional 30 minutes, the solid was
- 25 filtered off, resuspended in methanol (200 mL), stirred 5 minutes, filtered off, and dried in a vacuum oven.
- Poly(ethyleneimine sulfate C) - Intermediate "D" (1.0 g) was suspended in water (150 mL), stirred 30 minutes, and partially neutralized with sulfuric acid (0.28 g). The
- 30 mixture was stirred an additional 30 minutes, the solid was filtered off, resuspended in methanol (200 mL), stirred 5 minutes, filtered off, and dried in a vacuum oven.

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Poly(ethyleneimine sulfate D) - Intermediate "D" (1.0 g) was suspended in water (150 mL), stirred 30 minutes, and partially neutralized with sulfuric acid (0.11 g). The mixture was stirred an additional 30 minutes, the solid was
5 filtered off, resuspended in methanol (200 mL), stirred 5 minutes, filtered off, and dried in a vacuum oven.

Poly(ethyleneimine tartrate A) - Intermediate "D" (1.0 g) was suspended in water (150 mL), stirred 30 minutes, and partially neutralized with tartaric acid (1.72 g). The
10 mixture was stirred an additional 30 minutes, the solid was filtered off, resuspended in methanol (200 mL), stirred 5 minutes, filtered off, and dried in a vacuum oven.

Poly(ethyleneimine tartrate B) - Intermediate "D" (1.0 g) was suspended in water (150 mL), stirred 30 minutes, and
15 partially neutralized with tartaric acid (0.86 g). The mixture was stirred an additional 30 minutes, the solid was filtered off, resuspended in methanol (200 mL), stirred 5 minutes, filtered off, and dried in a vacuum oven.

Poly(ethyleneimine tartrate C) - Intermediate "D" (1.0 g)
20 was suspended in water (150 mL), stirred 30 minutes, and partially neutralized with tartaric acid (0.43 g). The mixture was stirred an additional 30 minutes, the solid was filtered off, resuspended in methanol (200 mL), stirred 5 minutes, filtered off, and dried in a vacuum oven.

25 Poly(ethyleneimine ascorbate A) - Intermediate "D" (1.0 g) was suspended in water (150 mL), stirred 30 minutes, and partially neutralized with ascorbic acid (4.05 g). The mixture was stirred an additional 30 minutes, the solid was filtered off, resuspended in methanol (200 mL), stirred 5
30 minutes, filtered off, and dried in a vacuum oven.

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Poly(ethyleneimine ascorbate B) - Intermediate "D" (1.0 g) was suspended in water (150 mL), stirred 30 minutes, and partially neutralized with ascorbic acid (2.02 g). The mixture was stirred an additional 30 minutes, the solid was
5 filtered off, resuspended in methanol (200 mL), stirred 5 minutes, filtered off, and dried in a vacuum oven.

Poly(ethyleneimine ascorbate C) - Intermediate "D" (1.0 g) was suspended in water (150 mL), stirred 30 minutes, and partially neutralized with ascorbic acid (1.01 g). The
10 mixture was stirred an additional 30 minutes, the solid was filtered off, resuspended in methanol (200 mL), stirred 5 minutes, filtered off, and dried in a vacuum oven.

Poly(ethyleneimine citrate A) - Intermediate "D" (1.0 g) was suspended in water (150 mL), stirred 30 minutes, and
15 partially neutralized with citric acid (1.47 g). The mixture was stirred an additional 30 minutes, the solid was filtered off, resuspended in methanol (200 mL), stirred 5 minutes, filtered off, and dried in a vacuum oven.

Poly(ethyleneimine citrate B) - Intermediate "D" (1.0 g) was suspended in water (150 mL), stirred 30 minutes, and
20 partially neutralized with citric acid (0.74 g). The mixture was stirred an additional 30 minutes, the solid was filtered off, resuspended in methanol (200 mL), stirred 5 minutes, filtered off, and dried in a vacuum oven.

Poly(ethyleneimine citrate C) - Intermediate "D" (1.0 g) was suspended in water (150 mL), stirred 30 minutes, and partially neutralized with citric acid (0.37 g). The
25 mixture was stirred an additional 30 minutes, the solid was filtered off, resuspended in methanol (200 mL), stirred 5
30 minutes, filtered off, and dried in a vacuum oven.

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Poly(ethyleneimine succinate A) - Intermediate "D" (1.0 g) was suspended in water (150 mL), stirred 30 minutes, and partially neutralized with succinic acid (1.36 g). The mixture was stirred an additional 30 minutes, the solid was
5 filtered off, resuspended in methanol (200 mL), stirred 5 minutes, filtered off, and dried in a vacuum oven.

Poly(ethyleneimine succinate B) - Intermediate "D" (1.0 g) was suspended in water (150 mL), stirred 30 minutes, and partially neutralized with succinic acid (0.68 g). The
10 mixture was stirred an additional 30 minutes, the solid was filtered off, resuspended in methanol (200 mL), stirred 5 minutes, filtered off, and dried in a vacuum oven.

Poly(ethyleneimine chloride) - Polyethyleneimine (100 g in 100 g water) was dissolved in water (640 mL additional) and
15 the pH was adjusted to 10 with concentrated HCl. Isopropanol (1.6 L) was added, followed by epichlorohydrin (19.2 mL). The mixture was stirred under nitrogen for 18 hours at 60°C. The solids were filtered off and rinsed with methanol (300 mL) on the funnel. The solid was rinsed
20 by resuspending it in methanol (4 L), stirring 30 minutes, and filtering off the solid. The rinse was repeated twice with methanol, followed by resuspension in water (1 gallon). The pH was adjusted to 1.0 with concentrated HCl, the solid was filtered off, resuspended in water (1
25 gallon), the pH again adjusted to 1.0 with concentrated HCl, the mixture stirred 30 minutes, and the solid filtered off. The methanol rinse was again repeated and the solid dried in a vacuum oven to yield 112.4 g.

Poly(dimethylethyleneimine chloride) - Poly(ethyleneimine
30 chloride) (5.0 g) was suspended in methanol (300 mL) and sodium carbonate (50 g) was added. Methyl iodide (20 mL) was added and the mixture heated to reflux for 3 days.

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Water was added to reach a total volume of 500 mL, the mixture stirred for 15 minutes, and the solid filtered off. The solid was suspended in water (500 mL), stirred 30 minutes, and filtered. The solid was suspended in water (1 L), the pH adjusted to 7.0 with concentrated HCl, and the mixture stirred for 10 minutes. The solid was filtered off, resuspended in isopropanol (1 L), stirred 30 minutes, filtered off, and dried in a vacuum oven to yield 6.33 g.

The Calcium Supplement

10 The calcium supplement can be any pharmaceutically acceptable calcium salt, such as calcium acetate (such as PhosLo® calcium acetate tablets), calcium carbonate (such as TUMS EX® tablets), calcium gluconate, calcium lactate, calcium levinulate, calcium citrate, calcium lactobionate
15 and calcium chloride. Preferably, the calcium supplement to be administered serves as both a calcium source and a buffering agent, such as calcium carbonate or calcium acetate. Alternatively the calcium supplement can be administered with calcium-rich foods.

20 The Administration

 The methods of the invention involve treatment of patients with hyperphosphatemia. Elevated serum phosphate is commonly present in patients with renal insufficiency, hypoparathyroidism, pseudohypoparathyroidism, acute
25 untreated acromegaly, overmedication with phosphate salts, and acute tissue destruction as occurs during rhabdomyolysis and treatment of malignancies.

 The invention also relates to the use of the calcium supplement(s) and the polymer(s) described herein for the
30 manufacture of a medicament or medicaments for the treatment of hyperphosphatemia.

 The term "patient" used herein is taken to mean any mammalian patient to which phosphate binders may be

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administered. Patients specifically intended for treatment with the methods of the invention include humans, as well as nonhuman primates, sheep, horses, cattle, goats, pigs, dogs, cats, rabbits, guinea pigs, hamsters, gerbils, rats and mice.

The polymers utilized in the methods of the inventions are generally orally administered in therapeutically effective amounts. Further, the polymer are preferably non-toxic and stable upon administration. A therapeutically effective amount of compound is that amount which produces a result or exerts an influence on the particular condition being treated. As used herein, a therapeutically effective amount of a phosphate binder means an amount which is effective in decreasing the serum phosphate levels of the patient to which it is administered.

By "non-toxic" it is meant that when ingested in therapeutically effective amounts neither the polymers nor any ions released into the body upon ion exchange are harmful or are substantially harmful.

By "stable" it is meant that when ingested in therapeutically effective amounts the polymers do not dissolve or otherwise decompose to form potentially harmful by-products, and remain substantially intact so that they can transport bound phosphate out of the body.

The present pharmaceutical compositions are generally prepared by known procedures using well known and readily available ingredients. In making the compositions of the present invention, the polymeric phosphate binder may be present alone, may be admixed with a carrier, diluted by a carrier, or enclosed within a carrier which may be in the form of a capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semi-solid or liquid material which acts as a vehicle, excipient or medium for the polymer. Thus, the compositions can be

-40-

in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, syrups, aerosols, (as a solid or in a liquid medium), soft or hard gelatin capsules, sterile packaged powders, and the like. Examples
5 of suitable carrier, excipients, and diluents include foods, drinks, lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, methyl cellulose,
10 methylhydroxybenzoates, propylhydroxybenzoates, propylhydroxybenzoates, and talc.

Likewise, the calcium supplement is, preferably, administered orally in an amount which is effective to provide a source of dietary calcium and the synergistic
15 effects described herein (e.g., the USDA recommended daily dosage or the complement thereof to the patients' normal calcium dietary intake due to food consumption). Thus, the administration increases the physiological calcium concentration in the patient and results in the superior
20 removal of phosphate.

Alternative modes of administration include intravenous administration (e.g., via an I.V. drip), administering calcium rich foods or an "ex vivo" administration, such as during dialysis.

25 The calcium supplement can be administered prior to, simultaneous with or subsequent to administration of the polymeric phosphate binder. In one embodiment, the calcium supplement administered can serve as a calcium source and a buffering agent, such as calcium carbonate or calcium
30 acetate, for the polymer.

The polymeric phosphate binder and the calcium supplement can be administered individually or as components of a single composition. For example, the calcium supplement can be included in one of the polymeric
35 phosphate binder formulations discussed above. In this

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case, the calcium supplement can additionally serve as a carrier or diluent for the polymeric phosphate binder. For example, the calcium supplement, such as calcium carbonate, can serve as a hardening agent in a tablet form of the
5 polymeric phosphate binder composition. Such a composition provides the polymeric phosphate binder, a calcium supplement and a carbonate supplement in a single dosage form.

10 Clinical Example of polymer/ calcium dietary supplement
coadministration

Hemodialysis patients (approximately 84 patients in about 6 centers) on stable doses of calcium and/or aluminum based phosphate binders entered a one to two week screening period. The administration of phosphate binders was
15 discontinued and, at the end of this period, the "washout phase". Patients who become very hyperphosphatemic (> 12 mg/dL) at any point during the washout phase were started on RenaGel® phosphate binder (polyallylamine crosslinked with epichlorohydrin) immediately. Patients who completed
20 the two week washout phase and developed hyperphosphatemia (serum $\text{PO}_4 > 6.0$ mg/dL) at either week 1 or week 2 were eligible for study drug treatment.

Eligible patients were randomly assigned to either RenaGel® phosphate binder alone or RenaGel® phosphate
25 binder with an evening calcium supplement. The RenaGel® phosphate binder starting dose was based on the degree of hyperphosphatemia. Starting doses were either six, nine, or twelve 465 mg capsules per day divided among meals to reflect the phosphate content of the meals. Patients
30 assigned to take an evening calcium supplement were instructed to take three TUMS EX® 750 mg tablets (900 mg of elemental calcium) on an empty stomach at bedtime.

At the end of each of three subsequent three weeks periods, the dose of RenaGel® phosphate binder was titrated

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up by three capsules per day as necessary to achieve a serum phosphate between 2.5 and 5.5 mg/dL, inclusive. Where the serum phosphate falls to less than 2.5 mg/dL, the investigator, using clinical judgment, decreased the
5 polymer dose by one to three capsules per day to elevate the serum phosphorus to above 2.5 mg/dL.

Where the serum calcium in a patient not randomized to receive calcium fell below normal (defined by the central laboratory normal range) during the study, an evening
10 calcium supplement was administered to the patient, starting with 3 TUMS EX® 750 mg tablets (900 mg of elemental calcium) as the carbonate salt on an empty stomach at bedtime and titrating upward as necessary.

At the conclusion of the treatment period, patients
15 were not administered any phosphate binder during a second washout phase. Upon completion this period, patients discontinued evening calcium supplements, where applicable, and returned to their original phosphate binders.

ASSESSMENTS AND PROCEDURES

20 Weekly throughout the trial, on Mondays (MWF patients) and Tuesdays (TTS patients), the patients gave blood for the laboratory studies just prior to receiving dialysis.

On the Wednesdays (MWF patients) and Thursdays (TTS patients) of the same weeks, the patients were asked to
25 report any adverse events or any changes in medications that may indicate adverse events. The results of the laboratory tests were also reviewed at this time.

Dietary intake of phosphate was assessed on randomly selected days within the first washout phase, the treatment
30 phase, and the second washout phase by the nutritionists at the treatment center.

PATIENT SELECTION CRITERIA

Approximately 84 (42 using Vitamin D and 42 not using Vitamin D) hemodialysis patients on stable doses of

-43-

phosphate binders entered the study. The patients had controlled serum phosphate and did not have any clinically significant unstable medical conditions. Only those patients who are hyperphosphatemic (serum $\text{PO}_4 > 6.0$ mg/dL) during the first washout period (approximately 72 patients) were selected. 36 patients (18 using Vitamin D and 18 not using Vitamin D) received RenaGel® phosphate binder alone and 36 (18 using Vitamin D and 18 not using Vitamin D) received RenaGel® phosphate binder with an evening calcium supplement.

STUDY AGENTS

RenaGel® phosphate binder was supplied as capsules containing 465 mg of polymer, 5 mg of colloidal silicon dioxide, and 5 mg of stearic acid. Each patient was started on one of three doses of RenaGel® phosphate binder divided among meals each day to reflect the phosphate content of the meals.

The assignment of a patient to a starting dose was made based on the highest serum phosphorus level observed during the first wash-out period.

- I. 6 capsules (2.79 g) per day with meals
- II. 9 capsules (4.19 g) per day with meals
- III. 12 capsules (5.58 g) per day with meals

A serum phosphorus of greater than 6.0 mg/dL and less than 7.5 mg/dL, greater than or equal to 7.5 mg/dL and less than 9.0 mg/dL, and greater than or equal to 9.0 mg/dL indicate starting dose groups I, II, and III, respectively.

The dose was titrated up three times during the twelve weeks in increments of 3 capsules per day. The maximum anticipated daily dose in this study was 9.8 g (21 capsules). If the dose needed to be reduced for low serum phosphorus ($\text{PO}_4 < 2.5$), the dose was decreased in increments of 1 to 3 capsules per day depending on the serum phosphorus level.

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The polymer was well tolerated. As illustrated in Figure 4, the administration of the calcium supplement with the phosphate binding polymer significantly reduced the serum phosphate levels in the patients. This amount
5 exceeds the amount which was expected due to phosphate-binding properties of the calcium supplement (Schiller, et al., *New England Journal of Medicine*, 320:1110-1113 (1989)). Figures 5 and 6 report the serum calcium and parathyroid hormone levels, respectively, of the two groups
10 of patients. Figure 7 illustrates the product of serum calcium levels times serum phosphate levels of the two patient groups. Patients receiving both polymer and calcium supplements had markedly lower products than patients receiving polymer alone. This product is an
15 indication of the soft-tissue calcification and is generally closely monitored upon the administration of calcium acetate as a phosphate binder (e.g., PhosLo® calcium acetate tablets) to ensure that the product does not exceed 66.

20 These results establish that the administration of a phosphate binding polymer and a calcium supplement are superior in the treatment of hyperphosphatemia.

It should be understood, however, that the foregoing description of the invention is intended merely to be
25 illustrative by way of example only and than other modifications, embodiments, and equivalents may be apparent to those skilled in the art without departing from its spirit.

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EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention
5 described herein. Such equivalents are intended to be encompassed by the following claims.

-46-

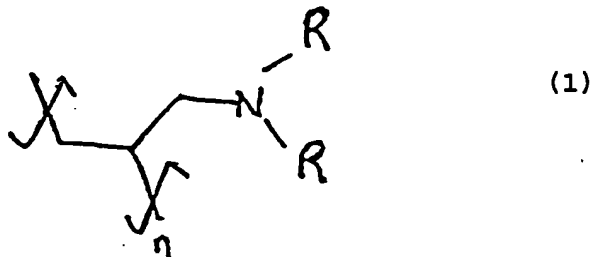
CLAIMS

What is claimed is:

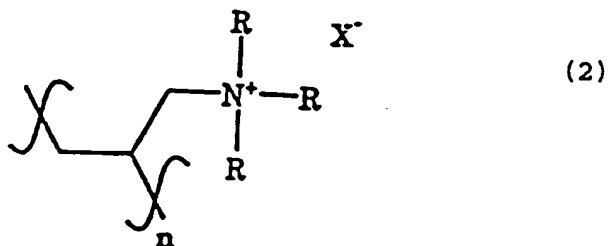
1. A method for removing phosphate from a patient and increasing the physiological calcium level of the patient, comprising administering to the patient a calcium supplement and a composition comprising at least one phosphate-binding polymer.
5
2. The method of Claim 1 wherein the calcium supplement is selected from the group consisting of calcium acetate, calcium carbonate, calcium gluconate, calcium lactate, calcium levinulate, calcium citrate, calcium lactobionate and calcium chloride.
10
3. A method for removing phosphate from a patient and increasing the physiological calcium level of the patient, comprising orally coadministering to the patient a therapeutically effective amount of a composition comprising at least one phosphate-binding polymer and a calcium supplement.
15
4. The method of Claim 3 wherein the calcium supplement is calcium acetate or calcium carbonate.
20
5. The method of Claim 3 wherein the calcium supplement is selected from the group consisting of calcium gluconate, calcium lactate, calcium levinulate, calcium citrate, calcium lactobionate and calcium chloride.
25
6. The method of Claim 4 wherein the calcium supplement is calcium carbonate and the composition is formulated as a tablet.

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7. The method of Claim 3 wherein the phosphate-binding polymer is characterized by a repeat unit having the formula



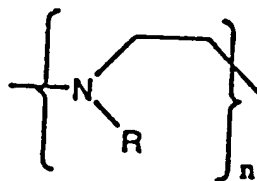
- 5 or a copolymer thereof, wherein n is an integer and each R is, independently, H or a substituted or unsubstituted alkyl, alkylamino or aryl group.
8. The method of Claim 3 wherein the phosphate-binding polymer is characterized by a repeat unit having the formula
- 10



or a copolymer thereof, wherein n is an integer and each R is, independently, H or a substituted or unsubstituted alkyl, alkylamino or aryl group.

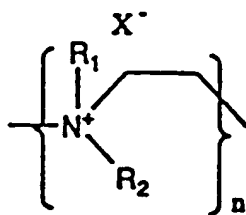
-48-

9. The method of Claim 3 wherein the phosphate-binding polymer is characterized by a repeat unit having the formula



(4)

- 5 or a copolymer thereof, wherein n is an integer and R is H or a substituted or unsubstituted alkyl, alkylamino or aryl group.
10. The method of Claim 3 wherein the phosphate-binding polymer is characterized by a repeat unit having the formula
- 10

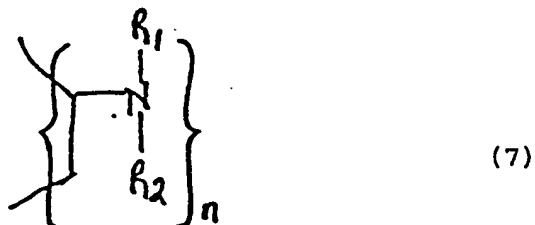


(6)

or a copolymer thereof, wherein n is an integer and R₁ and R₂ are each, independently, H or a substituted or unsubstituted alkyl, alkylamino or aryl group.

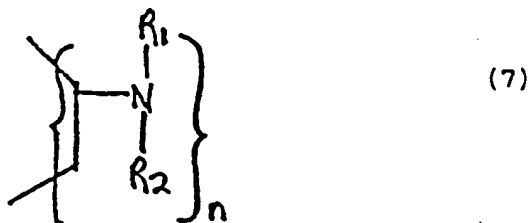
-49-

11. The method of Claim 3 wherein the phosphate-binding polymer is characterized by a repeat unit having the formula



- 5 or a copolymer thereof, wherein n is an integer and R_1 and R_2 are each, independently, H or a substituted or unsubstituted alkyl, alkylamino or aryl group.

12. The method of Claim 3 wherein the phosphate-binding polymer is characterized by a repeat unit having the formula
- 10

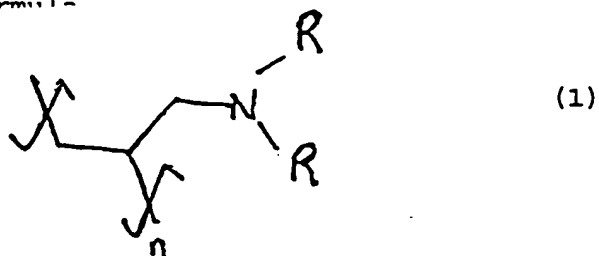


- or a copolymer thereof, wherein n is an integer and R_1 , R_2 and R_3 are each, independently, H or a substituted or unsubstituted alkyl, alkylamino or aryl group.
- 15

13. A composition comprising at least one phosphate-binding polymer and a calcium supplement.
14. The composition of Claim 13 wherein the supplement is calcium carbonate.

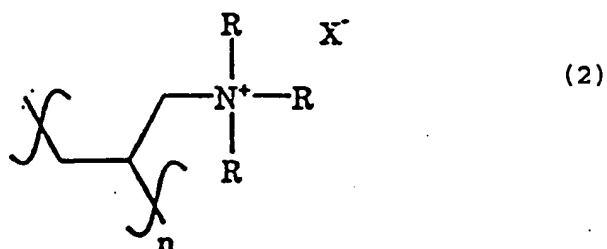
-50-

15. The composition of Claim 13 wherein the phosphate-binding polymer is characterized by a repeat unit having the formula-



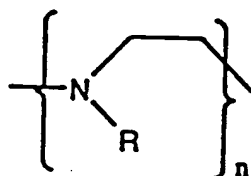
- 5 or a copolymer thereof, wherein n is an integer and each R is, independently, H or a substituted or unsubstituted alkyl, alkylamino or aryl group.

16. The composition of Claim 13 wherein the phosphate-binding polymer is characterized by a repeat unit having the formula
- 10



or a copolymer thereof, wherein n is an integer and each R is, independently, H or a substituted or unsubstituted alkyl, alkylamino or aryl group.

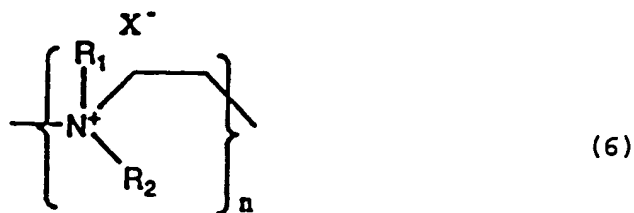
- 15 17. The composition of Claim 13 wherein the phosphate-binding polymer is characterized by a repeat unit having the formula



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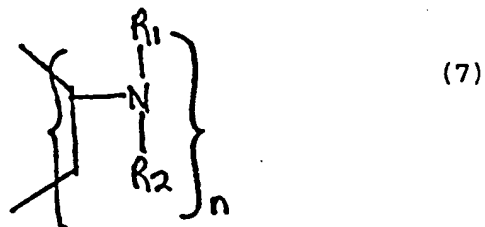
or a copolymer thereof, wherein n is an integer and R is H or a substituted or unsubstituted alkyl, alkylamino or aryl group.

18. The composition of Claim 13 wherein the phosphate-binding polymer is characterized by a repeat unit having the formula



- or a copolymer thereof, wherein n is an integer and R₁ and R₂ are each, independently, H or a substituted or unsubstituted alkyl, alkylamino or aryl group.

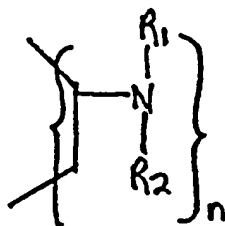
19. The composition of Claim 13 wherein the phosphate-binding polymer is characterized by a repeat unit having the formula



- or a copolymer thereof, wherein n is an integer and R₁ and R₂ are each, independently, H or a substituted or unsubstituted alkyl, alkylamino or aryl group.

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20. The composition of Claim 13 wherein the phosphate-binding polymer is characterized by a repeat unit having the formula



(7)

- 5 or a copolymer thereof, wherein n is an integer and R₁, R₂ and R₃ are each, independently, H or a substituted or unsubstituted alkyl, alkylamino or aryl group.

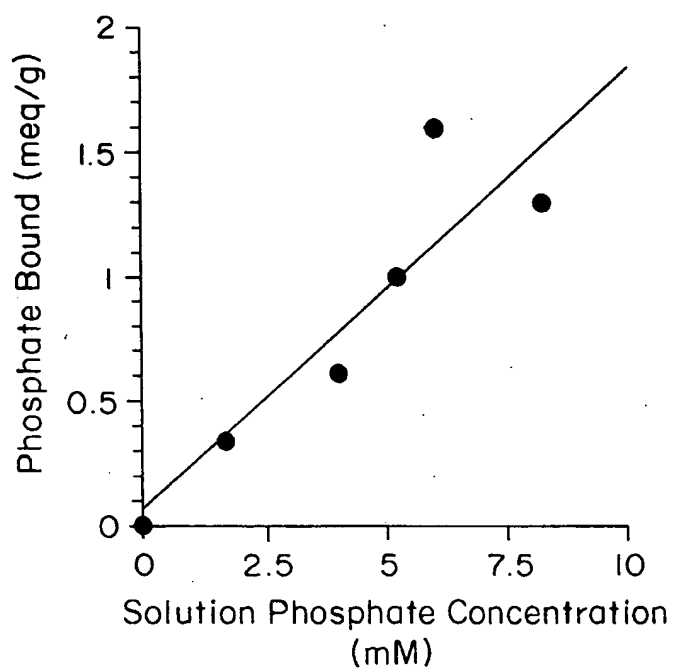


FIG. 1

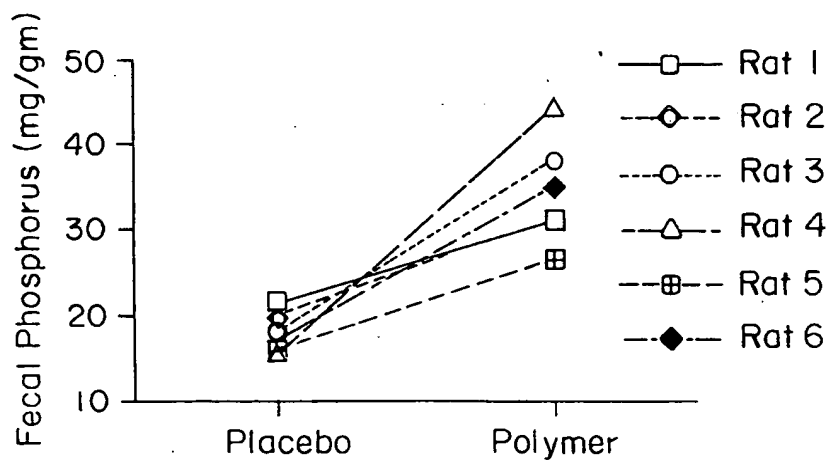
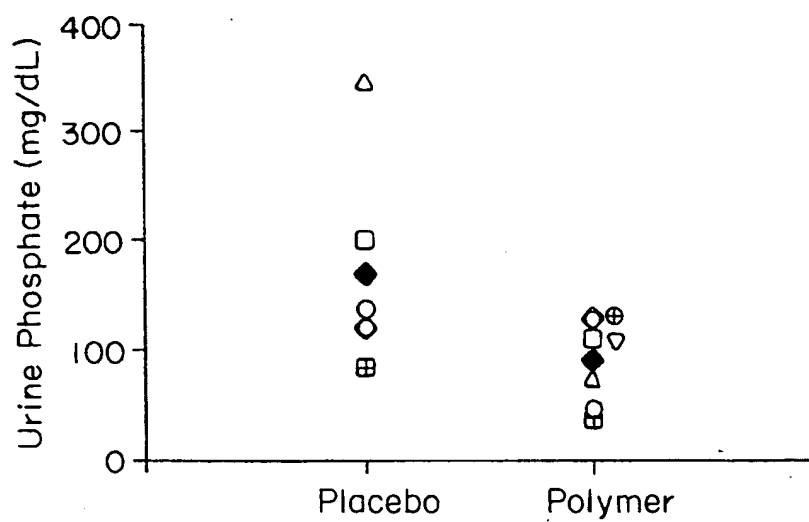


FIG. 2

**FIG. 3**

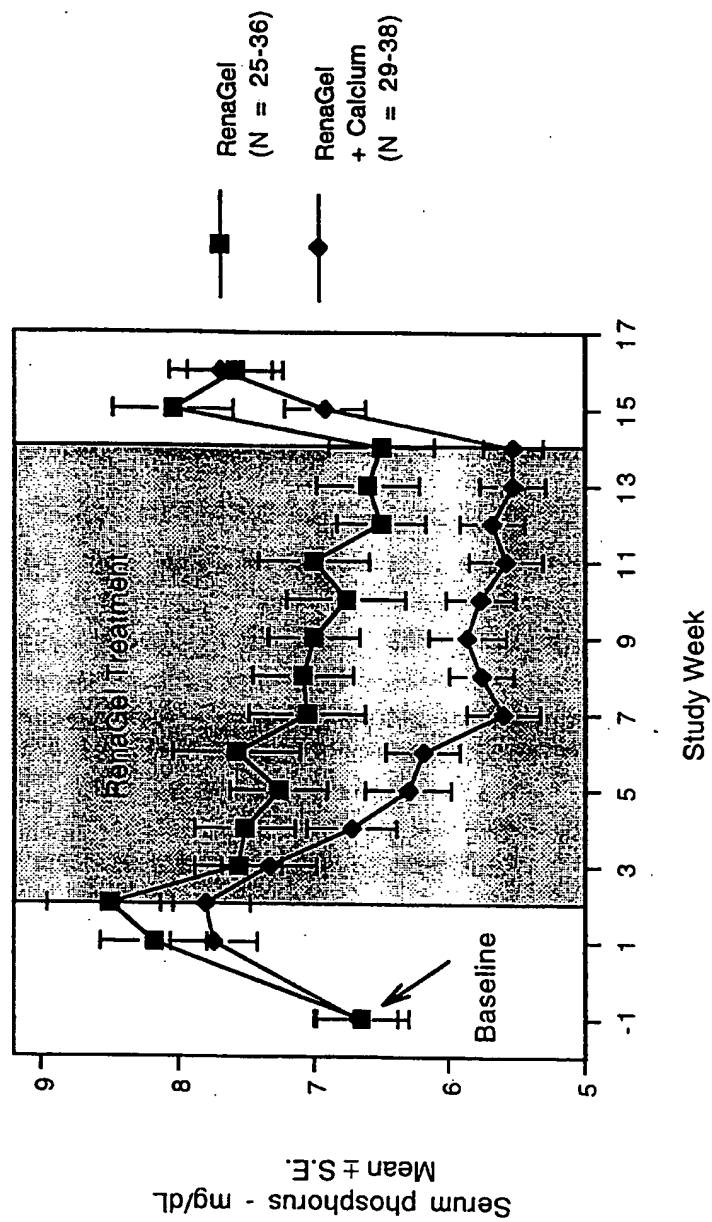


FIG. 4

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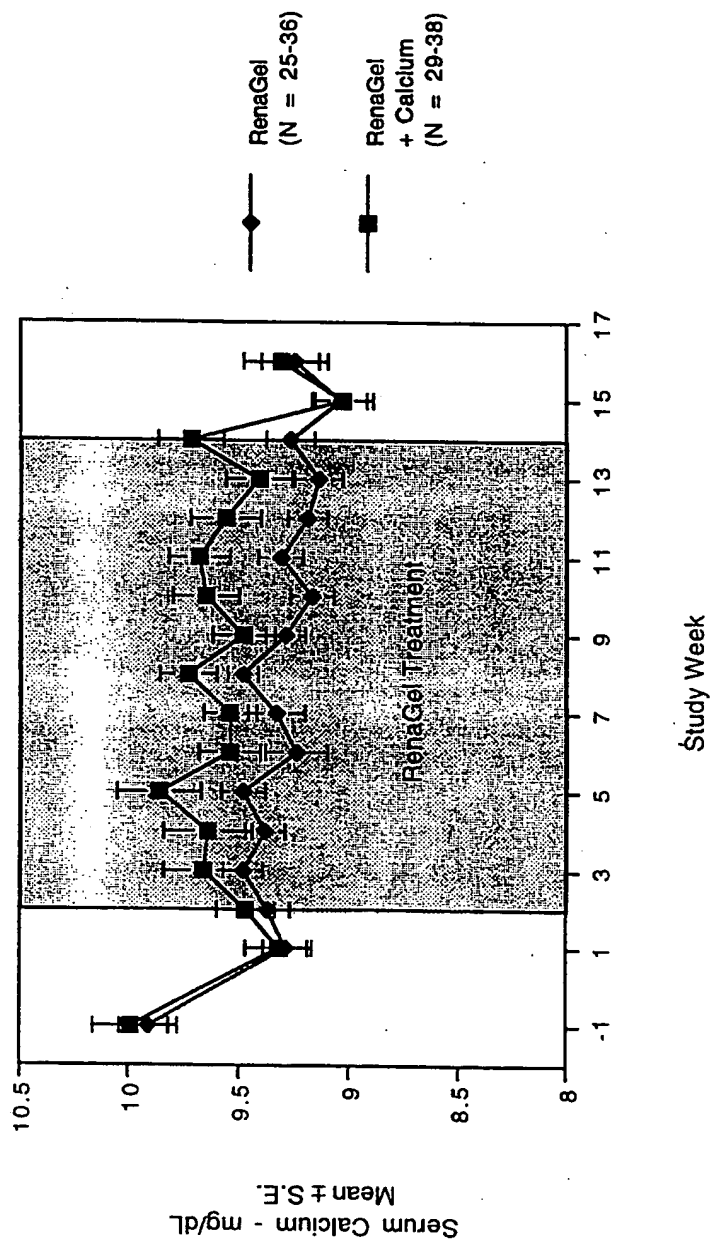


FIG. 5

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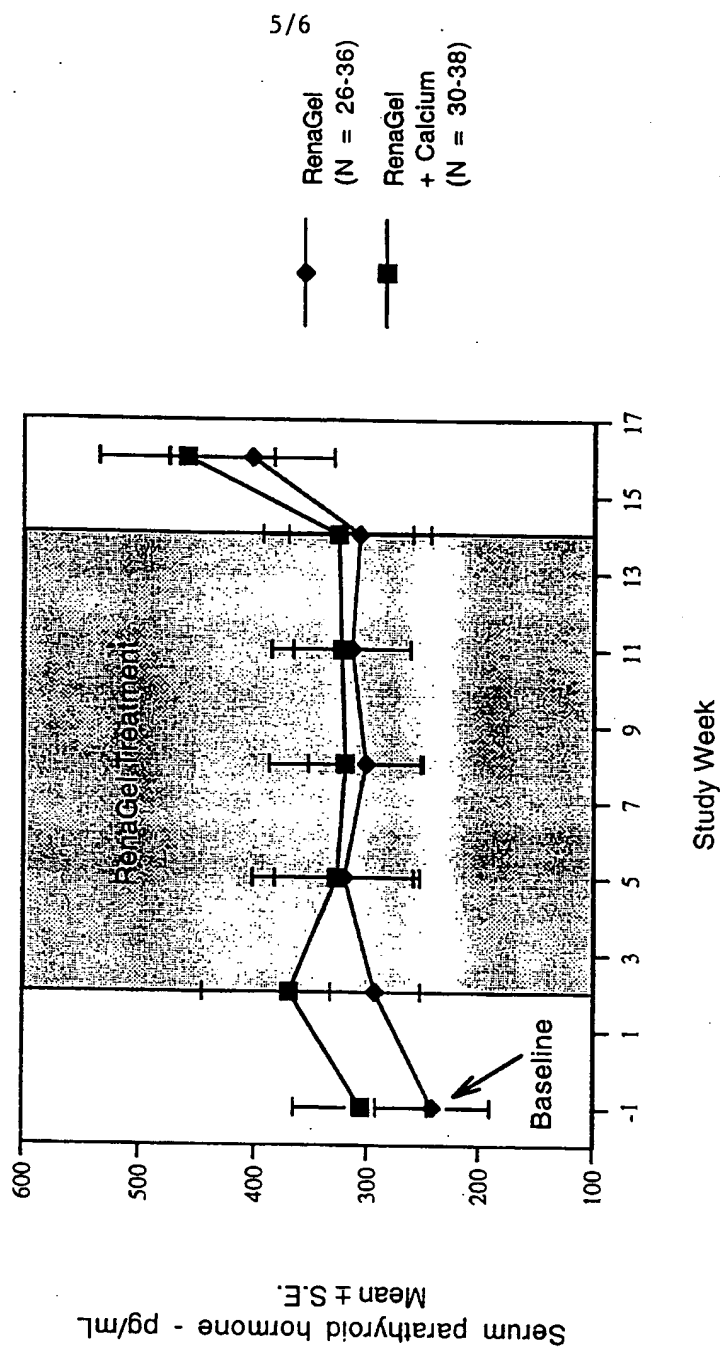


FIG. 6

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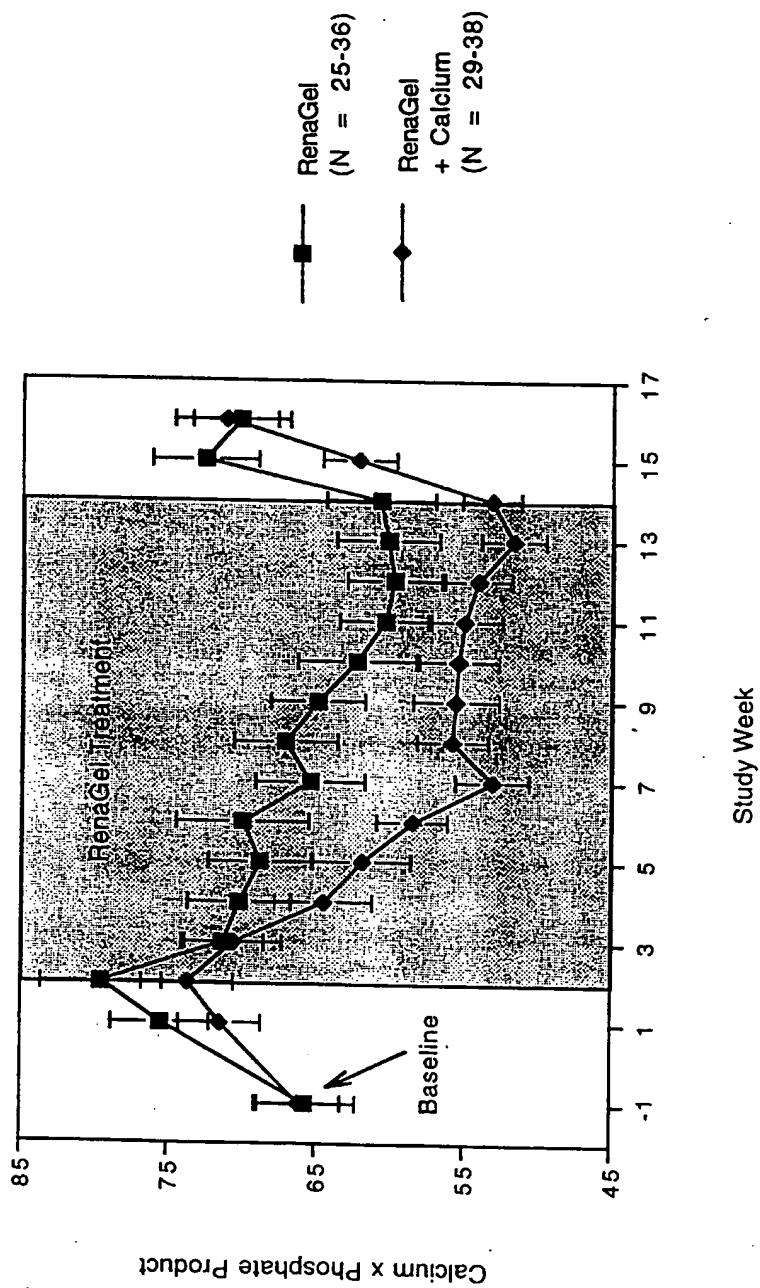


FIG. 7

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/05780

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K31/785 A61K33/10 A61K33/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR 2 391 730 A (BRISTOL MYERS CO) 22 December 1978 see page 1, line 21-29; claims 1,2 see page 4, line 24-30; examples ---	13,14,16
X	EP 0 605 757 A (AJINOMOTO KK) 13 July 1994 see page 3, line 39-43; claim 1; examples 4,6,9 ---	13-15
X	US 3 624 209 A (GRANATEK EDMUND S ET AL) 30 November 1971 see column 1, line 60-64; claims 1,4-6 see column 1, line 23-25 ---	13,14
A	WO 96 21454 A (GELTEX PHARMA INC) 18 July 1996 see page 1-2; claims see page 34-35 ---	1,2,4-20
	-/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *a* document member of the same patent family

Date of the actual completion of the international search

10 October 1997

Date of mailing of the international search report

28.10.97

Name and mailing address of the ISA

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Authorized officer

Kanbier, D

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/05780

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 05184 A (GELTEX PHARMACEUTICALS INC) 23 February 1995 see page 1-2; claims see page 17 see page 34-35 ---	1,2,4-20
A	WO 96 25440 A (BRITISH TECH GROUP) 22 August 1996 see claims 20-23; examples 8-10 ---	1,7-13, 15-20
A	GB 2 276 170 A (BRITISH TECH GROUP) 21 September 1994 see page 2-6; claims 15-18 -----	1,7-13, 15-20

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 97/05780

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 1-12
is(are) directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern 1st Application No

PCT/US 97/05780

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR 2391730 A	22-12-78	DE 2822546 A	07-12-78
		GB 1573487 A	28-08-80
		JP 54017133 A	08-02-79
		ZA 7802729 A	27-06-79

EP 0605757 A	13-07-94	JP 5316999 A	03-12-93
		US 5447732 A	05-09-95

US 3624209 A	30-11-71	NONE	

WO 9621454 A	18-07-96	NONE	

WO 9505184 A	23-02-95	US 5496545 A	05-03-96
		AU 7560794 A	14-03-95
		EP 0716606 A	19-06-96
		JP 9504782 T	13-05-97
		US 5667775 A	16-09-97

WO 9625440 A	22-08-96	AU 4673296 A	04-09-96

GB 2276170 A	21-09-94	AU 6009294 A	14-09-94
		CA 2153151 A	01-09-94
		EP 0684958 A	06-12-95
		WO 9419379 A	01-09-94
		JP 8506846 T	23-07-96
